AD-A214 031

AD _____

HOST DEFENSE AGAINST OPPORTUNIST MICROORGANISMS FOLLOWING TRAUMA

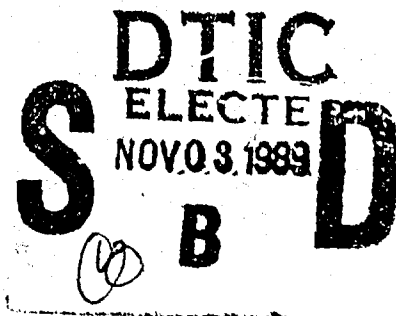Annual Report

June 30, 1989

DTIC FILE COPY

Ann B. Bjornson, Ph.D.
H. Stephen Bjornson, M.D., Ph.D.

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-87-C-7165

DTIC
ELECTE
NOV.0.3.1989
S B D

James N. Gamble Institute of Medical Research
Cincinnati, Ohio 45219

Approved for public release; distribution unlimited.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	Approved for public release; distribution unlimited.

4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION James N. Gamble Institute of Medical Research	6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) Cincinnati, Ohio 45219		7b. ADDRESS (City, State, and ZIP Code)

8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-87-C-7165

8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS			
	PROGRAM ELEMENT NO. 62787A	PROJECT NO. 3S1 62787A874	TASK NO. CH	WORK UNIT ACCESSION NO. 280

11. TITLE (Include Security Classification)

(U) Host Defense Against Opportunist Microorganisms Following Trauma

12. PERSONAL AUTHOR(S)
Bjornson, A. B., Bjornson, H. S.

13a. TYPE OF REPORT Annual	13b. TIME COVERED FROM 7/1/88 TO 6/30/89	14. DATE OF REPORT (Year, Month, Day) 1989, June 30	15. PAGE COUNT 41

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	thermal injury; trauma; host resistance; infection; immunosuppression; polymorphonuclear leukocyte function; arachidonic acid metabolism; RA II
06	05		
06	13		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Prostaglandins of the E series inhibit major effector functions of polymorphonuclear leukocytes (PMNs) by elevating intracellular cyclic-3',5'-adenosine monophosphate (cAMP). The present study investigated the involvement of this mechanism in the bactericidal defect of PMNs induced by thermal injury in a guinea pig model. Peripheral and peritoneal exudate PMNs harvested from thermally injured guinea pigs at one or two days postburn had decreased bactericidal activity against *Pseudomonas aeruginosa* and a marked increase in cAMP content. Production of prostaglandin E_1 (PGE_1) by these cells in the absence of exogenous PMN activators was also increased. Treatment of PMNs in vitro or in vivo with nonsteroidal anti-inflammatory drugs (NSAIDs; indomethacin, ibuprofen, and piroxicam) restored bactericidal activity to normal and concomitantly reduced cAMP content and PGE_1 production. A concomitant reduction in cAMP content and PGE_1 production was also ob-
continued on next page

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Virginia M. Miller	22b. TELEPHONE (Include Area Code) 301/663-7325	22c. OFFICE SYMBOL SGRD-RMI-S

19. continued

served as bactericidal activity of PMNs returned to normal under natural conditions during four to seven days postburn. The enhancement of PMN bactericidal activity mediated by NSAIDs was fully counteracted by purified PGE_1, theophylline, and by cAMP itself. These results suggest that the bactericidal defect of PMNs induced by thermal injury is related to elevation of cAMP and that PGE_1 plays a significant role in this phenomenon.

FOREWORD

In conducting the research described in this report, the investigators adhered to the Guide for the Care and Use of Laboratory Animals, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 86-23, Revised 1985). Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

BODY OF REPORT

Statement of the Problem under Study

Sepsis continues to be a leading cause of morbidity and mortality in thermally injured patients despite recent improvements in the clinical care of these patients [1]. Susceptibility to bacterial infection is increased because of loss of the protective skin barrier and the profound depression of host defense mechanisms induced by severe injury [reviewed in 2]. Poly-morphonuclear leukocytes (PMNs) are critically important for maintaining the sterility of tissue spaces and for facilitating clearance of invading bacteria from circulation [3]. Major effector functions of these cells are markedly reduced by thermal injury including chemotaxis, oxidative metabo-lism, and bactericidal activity [4-13]. Several studies have suggested that the bactericidal defect of PMNs in thermally injured patients contri-butes to the development of life-threatening infections [4,6,7]. Despite the probable importance of PMN dysfunction in the predisposition to bacter-ial infection associated with thermal injury, little is known about the mechanisms responsible for this abnormality.

Recent studies have suggested that PMNs are initially activated in response to thermal injury [12]. However, the biochemical events occurring after these cells are activated that lead to the down regulation of criti-cal effector functions are totally unknown. Studies from our laboratory have suggested that arachidonate metabolites of the cyclooxygenase pathway may be involved in this phenomenon. Using a guinea pig model of thermal injury, we demonstrated that the bactericidal activity of PMNs is depressed in temporal association with increased generation of prostaglandins and thromboxane in transudate at the site of thermal injury [14]. In addition, we showed that selective inhibition of the cyclooxygenase pathway by paren-

6

teral therapy with NSAIDs corrects the bactericidal defect of PMNs in the thermally injured animals [15].

Prostaglandins of the E series are known to inhibit various effector functions of PMNs including chemotaxis, aggregation, superoxide production, and lysosomal enzyme release [16-23]. These prostaglandins exert their effect by acting as adenyl cyclase agonists, raising intracellular cyclic-3',5'-adenosine monophosphate (cAMP) to an abnormally high level [16-18,20]. It has been suggested that elevation of cAMP serves as an important signal for the cell to stop functioning, replenish energy supplies, and replace the surface membrane. This normal regulatory mechanism may be called into play in response to the heightened activation of PMNs induced by thermal injury resulting in down regulation of effector functions. The present investigation was undertaken to test this hypothesis. Nonsteroidal anti-inflammatory drugs (NSAIDs), prostaglandins of the E series, theophylline, and cAMP were used as tools to modulate the bactericidal activity of PMNs from thermally injured animals. Through this approach and by measuring the cAMP content of the PMNs under identical conditions, we provide evidence that the bactericidal defect of PMNs induced by thermal injury is related to a marked elevation of cAMP.

Methods

Animals. Male and female Hartley guinea pigs weighing 300 to 350 g were purchased from Murphy Breeding Laboratories, Inc., Plainfield, IN. The animals were housed in separate cages and adapted to the new environment for four to five days. The animals were fed guinea pig chow ad libitum.

Experimental thermal injury. Animals were anesthetized by intraperitoneal injection of 30-33 mg/kg sodium pentobarbital (Butler & Co., Columbus, OH) supplemented with inhaled methoxyflurane (Pitman-Moore, Washington

Crossing, NJ) as needed. Failure to flinch in response to pinching of the skin was used as the endpoint to judge that the animals were adequately anesthetized. Full thickness scald burns covering thirty percent of the total body surface were applied as previously described [14]. Fifteen ml of lactated Ringer's solution (Abbott Laboratories, North Chicago, IL) was administered intraperitoneally before and at 1.5 h after injury. Immediately after injury, the animals were placed on heating blankets (set on low) to reduce heat loss and thereby minimize stress. After the second dose of Ringer's solution, the animals were replaced in their cages. Sham-treated animals were used in some experiments. These animals were anesthetized and resuscitated as described above and immersed in tepid water to simulate burning.

Preparation of PMNs. Animals were anesthetized as described above, and blood for preparation of peripheral PMNs was drawn by cardiac puncture into heparinized syringes (50 units of heparin/ml of blood). Blood from two to five animals in each treatment group was pooled in order to yield sufficient numbers of leukocytes to conduct the experiments. One volume of heparinized blood was mixed with two volumes of 3% dextran (200,000-275,000 mol wt; Sigma Chemical Co., St. Louis, MO) in 0.15 M sodium chloride and allowed to sediment for 90 min at $4^{\circ}C$. For bactericidal assays, PMNs were further purified by centrifugation through lymphocyte separation medium (Organon Teknika Corp., Durham, NC) according to McCarthy et al. [24]. For measurements of cAMP content and PGE_1 production, PMNs were further purified by a minor modification of the method of Zimmerli et al. [25]; this method was used, because final preparations contained no contaminating erythrocytes (an artifactual source of cAMP). Three ml of supernatant from the dextran sedimentation was layered onto 4 ml of a 39%/65% Percoll gradient prepared in Hank's balanced salt solution (HBSS; M.A. Bioproducts,

8

Walkerville, MD). The gradients were centrifuged at 350 g for 25 min at $4^{\circ}C$. PMNs accumulated at the interface were removed and washed once with HBSS. Following both techniques, erythrocytes were lysed with 0.2% sodium chloride for 20 s; isotonicity was restored by addition of an equal volume of 1.6% sodium chloride. The cells were resuspended in Hank's balanced salt solution containing 0.1% gelatin (HBG) for bactericidal assays and HBSS for measurement of superoxide anion (O_2^-) production. For measurements of cAMP and prostaglandin E_1 (PGE_1) production, PMNs were resuspended in HBSS containing 20 mM Hepes (Sigma Chemical Co.), pH 7.4. Final preparations contained $\geq$ 85% PMNs and $\leq$15% mononuclear cells.

In some experiments, PMNs were obtained from peritoneal exudates. The cells were elicited by intraperitoneal injection of 10 ml of 15% casein (wt/vol) in sterile 0.15 M sodium chloride, pH 7.0 [26]. The peritoneal cavity was lavaged 18 h later with 50 ml of HBSS. Peritoneal exudates from two to three animals were pooled, and PMNs were purified by centrifugation through lymphocyte separation medium and hypotonic lysis as described above. These preparations contained >95% PMNs.

Measurement of bactericidal activity. A clinical isolate of *Pseudomonas aeruginosa* strain Wk, was grown for 4 h at $37^{\circ}C$ in trypticase soy broth (BBL, Cockeysville, MD). The bacteria were washed and resuspended in 0.01 M phosphate buffered saline, pH 7.4. PMN bactericidal activity was measured by enumerating total surviving bacteria by colony counting [27]. Equal parts of bacteria (5.0×10^8 cfu/ml) and 50% pooled normal guinea pig serum (vol/vol) were incubated for 15 min at $37^{\circ}C$. The bacteria were then washed once and resuspended in HBG. Opsonized bacteria (1.0×10^7 cfu/ml) and 5.0×10^6 leukocytes/ml (bacteria to leukocyte ratio of 2:1) were rotated for 60 min at $37^{\circ}C$. In some experiments, the number of opsonized bacteria was increased to 5.0×10^7 cfu/ml or 2.5×10^8 cfu/ml yielding bacteria to

9

leukocyte ratios of 10:1 and 50:1 respectively. Fifty μl samples were removed immediately before incubation and after 15, 30, and 60 min of incubation. The samples were serially diluted in distilled water, and the dilutions were plated on MacConkey agar (Difco Laboratories, Detroit, MI). Percent killing was calculated by the formula (a-b)/a x 100, where a and b were equal to surviving cfu/ml before and after incubation respectively.

Measurement of O_2^- production. A minor modification of the method of Fantone et al. [22] was used to measure O_2^- production by peripheral PMNs. Five hundred μl of leukocyte suspension (4.0 x 10^6 cells/ml) was preincubated for 5 min at 37°C with 340 μl of 0.23 mM ferricytochrome c, 10μl of 500 μg/ml cytochalasin B, and 50 μl of HBSS with and without 1 mg/ml superoxide dismutase (SOD). One hundred μl of 10^{-4} M formylmethionylleucyl-phenylalanine (FMLP) was then added, and the reactants were further incubated for 20 min at 37°C. The reaction was stopped by the addition of 500μl of HBSS containing 1 mg/ml SOD to tubes lacking SOD and 500 μl of HBSS alone to tubes containing SOD. The tubes were centrifuged at 700g for 5 min at 4°C, and the absorbance of the supernatants was read at 550 nm. The amount of O_2^- produced was calculated from the difference in absorbance of the samples with and without SOD in the reaction using a mM extinction coefficient for cytochrome c of 21.1.

Determination of cAMP content. One ml samples containing 5.0 x 10^6 leukocytes (untreated or treated with NSAIDs and/or cAMP activators as described below) were boiled for 5 min and then sonicated with a virsonic cell disruptor (Virtis Co., Gardner, NY). The cells were centrifuged at 1,000 g for 10 min at 4°C, and the supernatants were assayed for cAMP using a cAMP radioimmunoassay kit from New England Nuclear (Boston, MA). Samples were acetylated and assayed according to the manufacturer's recommended procedure. Greater than 90% of the cAMP reactivity in PMN supernatants

10

from normal and injured animals was destroyed by incubation for 30 min at 30°C with 0.0005 units/ml of beef heart phosphodiesterase (Sigma Chemical Co.).

Spontaneous production of PGE_1 by PMNs. One ml samples containing 3.0 x 10^7 leukocytes (untreated or treated with NSAIDs as described below) were incubated for 15 min at 37°C in the absence of exogenous PMN activators. The cells were centrifuged at 750 g for 3 min at 4°C, and the supernatants were removed. Prostaglandins were extracted from the supernatants by the method of Powell [28] using Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA). Solvent was evaporated under nitrogen flow, and the dried residue was dissolved in radioimmunoassay buffer. PGE_1 was assayed according to the manufacturer's recommended procedure using a kit from Advanced Magnetics (Cambridge, MA). According to the manufacturer, the antiserum to PGE_1 in the kit was 100% cross reactive with PGE_1, 9% cross reactive with PGE_2, and less than 1% cross reactive with other major arachidonate metabolites. A preliminary experiment was conducted to assess the procedural loss through the extraction procedure. Approximately 10,000 cpm of [^{3}H]PGE_1 (Advanced Magnetics) was applied in duplicate to the cartridges and extracted under identical conditions to the PMN supernatants. The procedural loss (mean ± SEM) was 20 ± 1% based on the recovery of radioactivity as compared with the input. Final PGE_1 concentrations were corrected for this procedural loss.

Preparation of NSAIDs. Indomethacin (Sigma Chemical Co.) and piroxicam (Pfizer, Westpoint, PA) were solubilized in 0.2 M sodium phosphate buffer, pH 7.4, at a concentration of 7.5 mg/ml by dropwise addition of 2 N sodium hydroxide to pH 8.5. The solutions were then titrated back to pH 7.4 with 6 N hydrochloric acid. Ibuprofen (50 mg/ml) was obtained from the Upjohn Co., Kalamazoo, MI. For in vitro experiments, the drugs were diluted to 2

11

mM in 0.2 M sodium phosphate buffer, pH 7.4, before use. The drugs were prepared fresh daily and used immediately.

Treatment of PMNs with NSAIDs in vitro. Leukocytes (1.0×10^7 cells/ml) were preincubated with indomethacin, ibuprofen, or piroxicam for 15 min at room temperature before addition to the assays. Preliminary experiments demonstrated that preincubation of PMNs from normal or injured animals under these conditions in the absence of NSAIDs had no effect on bactericidal activity, cAMP content, or PGE_1 production. Concentrations of NSAIDs used in the experiments are specified in the Results section.

Treatment of PMNs with piroxicam in vivo. In experiments in which the effects of piroxicam in vivo were determined, injured animals were injected intramuscularly with 15 mg/kg of piroxicam at 3 h postburn. In experiments in which PMNs were harvested at two days postburn, the injured animals were injected intramuscularly with a second dose of piroxicam (15 mg/kg) at approximately 18 h postburn.

Addition of cAMP activators or cAMP to assays. In experiments in which cAMP activators or cAMP were tested, these reagents were added to the assays together with normal PMNs or PMNs from injured animals that had been treated with piroxicam in vitro or in vivo as described above. Piroxicam remained in the assays with the cAMP activators or cAMP. The sources of these reagents were as follows: PGE_1 (Advanced Magnetics), PGE_2 (two different preparations, one each from Advanced Magnetics and Upjohn Diagnostics), theophylline (Sigma Chemical Co.), cAMP (Sigma Chemical Co.), and dibutyryl cAMP (Sigma Chemical Co.). For determination of cAMP content, PMNs (5.0×10^6 cells/ml) were incubated with PGE_1, PGE_2, or theophylline for 2 min at $37^{\circ}C$ before boiling. Concentrations of cAMP activators and cAMP used in the experiments are specified in the Results section.

12

Results

Alterations in bactericidal activity and O_2^- production by PMNs follow-
ing thermal injury. Our previous studies have demonstrated that the
bactericidal activity of peripheral PMNs against *P. aeruginosa* is depressed
in thermally injured guinea pigs during the first four days postburn and
returns to normal by seven days postburn [14,15]. We have also shown that
phagocytosis of *P. aeruginosa* by PMNs from the injured animals in the pre-
sence of normal serum occurs normally during this period, suggesting that
the bactericidal defect reflects only the efficiency of intracellular kill-
ing and not an abnormality in phagocytosis [14,15]. In the present study,
we attempted to determine whether the postburn bactericidal defect was
affected by varying the ratio of PMNs to bacteria in the assay. In these
experiments, peripheral PMNs were harvested from injured animals at one day
postburn, the time when bactericidal activity was shown to be maximally
reduced in our previous studies [14,15]. Bacteria to leukocyte ratios of
50:1, 10:1, and 2:1 were employed in the assay. The postburn bactericidal
defect was demonstrable under all three sets of conditions; however, the
defect was greatest at bacteria to leukocyte ratios of 10:1 and 2:1 (Fig.
1). The 2:1 ratio was used in subsequent studies.

To determine if the postburn bactericidal defect was associated with an
abnormality in oxidative metabolism, FMLP-induced O_2^- production by PMNs
from injured and normal animals was compared. O_2^- production by PMNs from
injured animals was reduced during three days postburn and returned to
normal by seven days postburn (Fig. 2). These results taken together with
the results of our previous studies indicated that bactericidal activity
and O_2^- production by PMNs were depressed concomitantly in this experimen-
tal model.

13
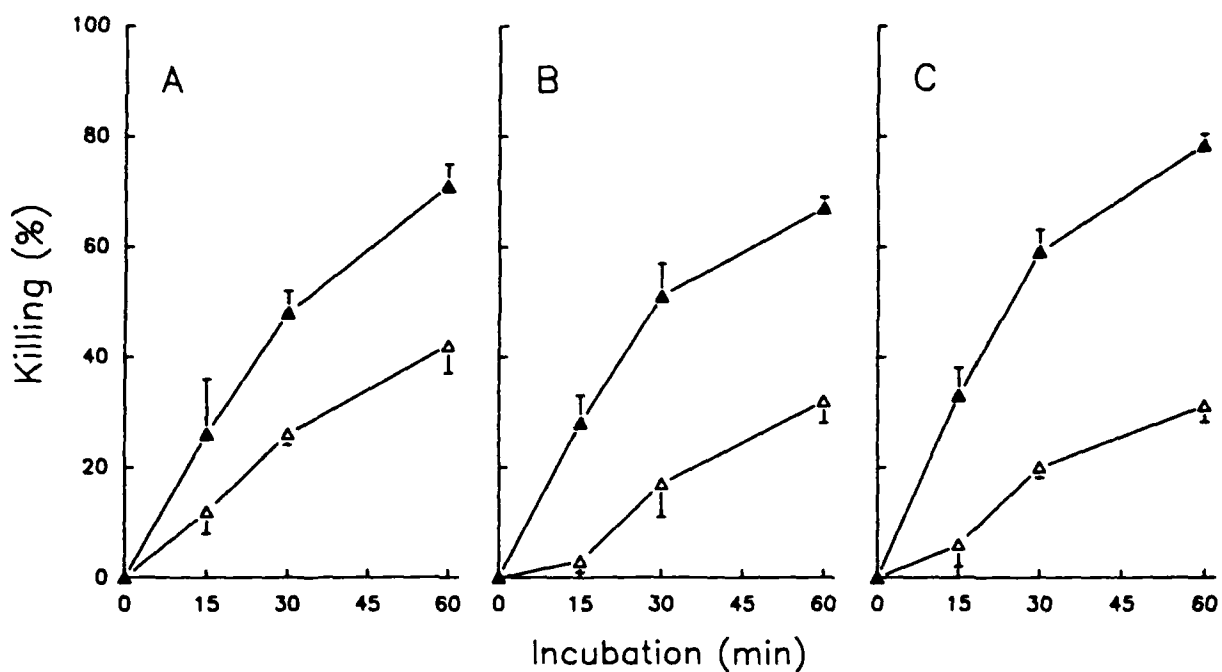
Figure 1. Effect of varying the bacteria to leukocyte ratio on the post-burn bactericidal defect of PMNs. PMNs obtained from injured animals (△) at one day postburn and from normal animals (▲) were assayed at bacteria to leukocyte ratios of 50:1 (A), 10:1 (B), and 2:1 (C). Data are mean ± SEM from three separate experiments.

Figure 2. Effect of thermal injury on O_2^- production by PMNs in response to FMLP. The open, boxed area shows the range of O_2^- production by PMNs from sham-treated animals (mean $\pm$ SD) in five separate experiments.

Direct effect of NSAIDs on the bactericidal activity of PMNs. Our previous studies demonstrated that indomethacin, ibuprofen, and piroxicam restored the bactericidal activity of PMNs to normal when administered in vivo to thermally injured anim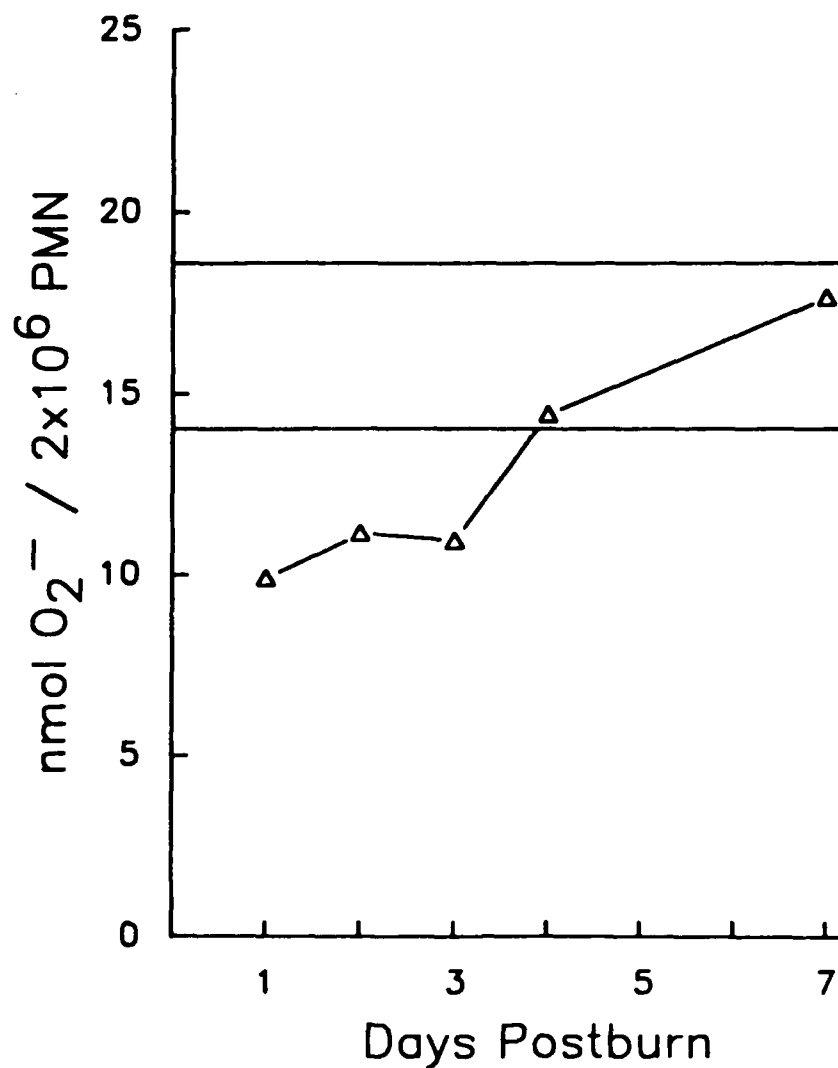als [15]. To determine if this effect was related to a direct interaction of the NSAIDs with the PMNs, the effect of these drugs on PMN bactericidal activity was determined in vitro. In these and subsequent experiments, PMNs were harvested from injured animals at one or two days postburn. NSAIDs markedly enhanced the bactericidal activity of PMNs from injured animals (Fig. 3) without affecting the bactericidal activity of normal PMNs (Fig. 4).

Additional studies investigated the concentrations of NSAIDs and conditions required for maximal enhancement of the bactericidal activity of PMNs from injured animals. A concentration of 100 μM NSAID was required to fully restore bactericidal activity to normal; results using piroxicam and indomethacin are shown in Fig. 5. Maximal enhancement of bactericidal activity was achieved when the NSAIDs were preincubated for 15 min at room temperature with the PMNs before assay rather than merely being added to the PMNs without preincubation. Therefore, in all subsequent experiments, PMNs were preincubated with 100 μM NSAID under these conditions before assay.

Effect of PGE_1 and PGE_2 on the bactericidal activity of PMNs. In the experiments described above, NSAIDs may have enhanced the bactericidal activity of PMNs from injured animals by inhibiting production of E type prostaglandins by these cells. If the observed effects were related to this mechanism, then addition of exogenous PGE_1 or PGE_2 to the assays should counteract the enhancement of bactericidal activity mediated by NSAIDs. To test this hypothesis, PMNs from injured animals were treated with piroxicam in vitro or in vivo, and the bactericidal activity of these

16

Figure 3. Effect of NSAIDs on the bactericidal activity of PMNs from thermally injured animals. PMNs were harvested from injured animals at one (A) or two (B) days postburn. The cells were preincubated for 15 min at room temperature with 100 μM indomethacin (O), ibuprofen (■), or piroxicam (●), or received no treatment (△) before addition to the assay. PMNs from normal animals (□) were untreated. Data are from a representative experiment run in duplicate. The difference between duplicates in these and subsequent experiments was usually less than 10%.
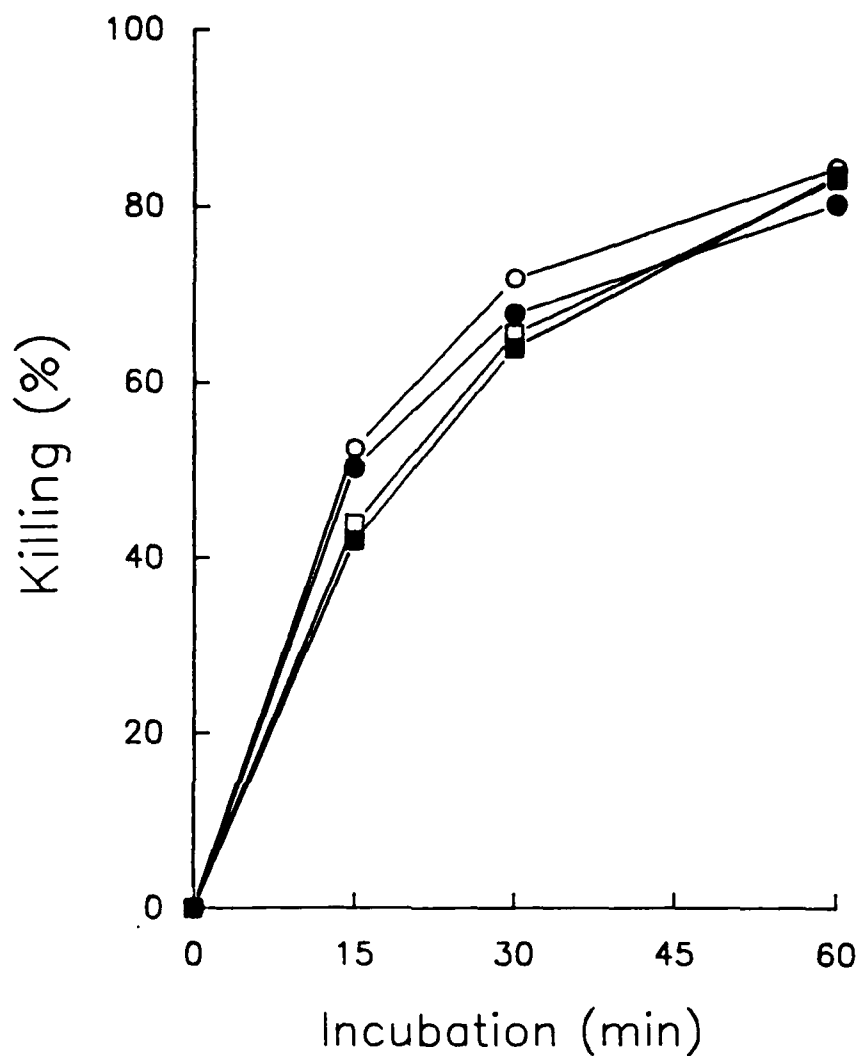
Figure 4. Effect of NSAIDs on the bactericidal activity of PMNs from normal animals. Normal PMNs were preincubated for 15 min at room temperature with 100 μM indomethacin (O), ibuprofen (■), or piroxicam (●), or received no treatment (□) before addition to the assay. Data are from a representative experiment run in duplicate.
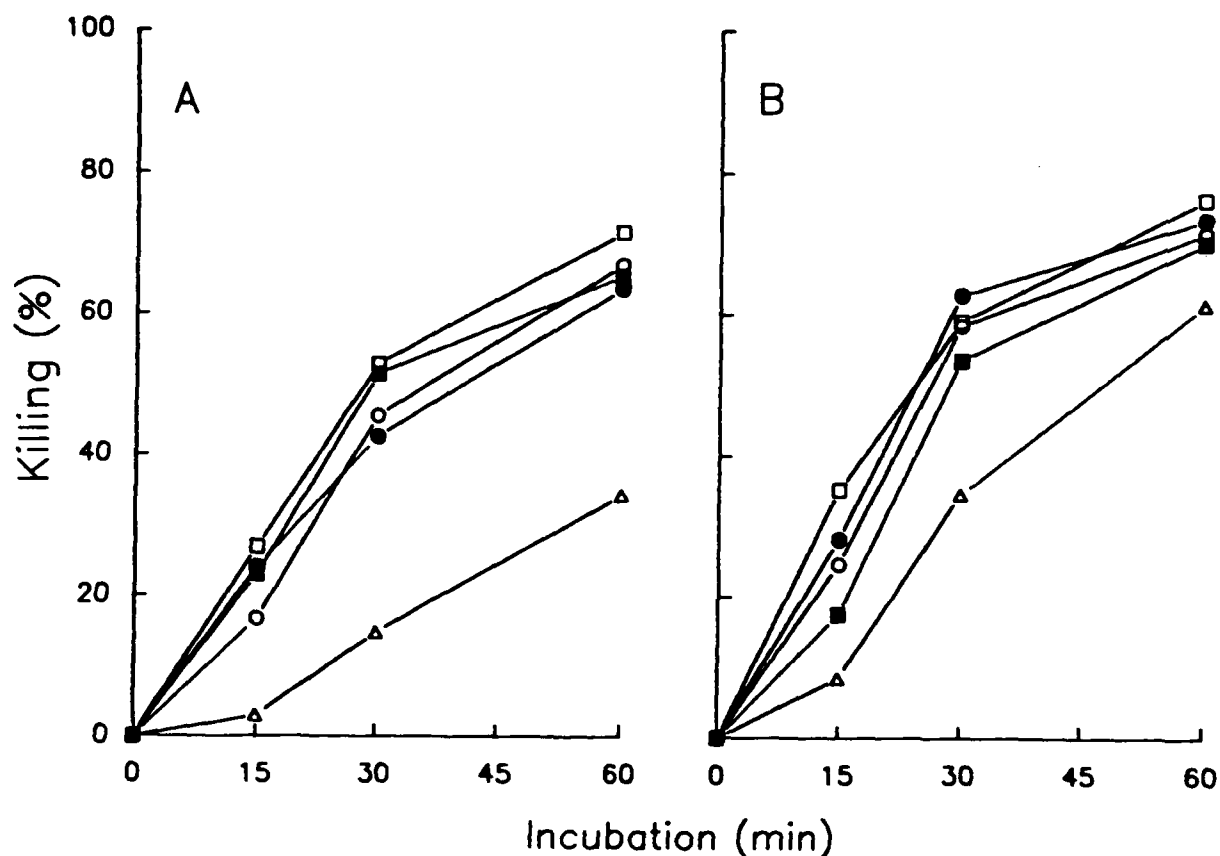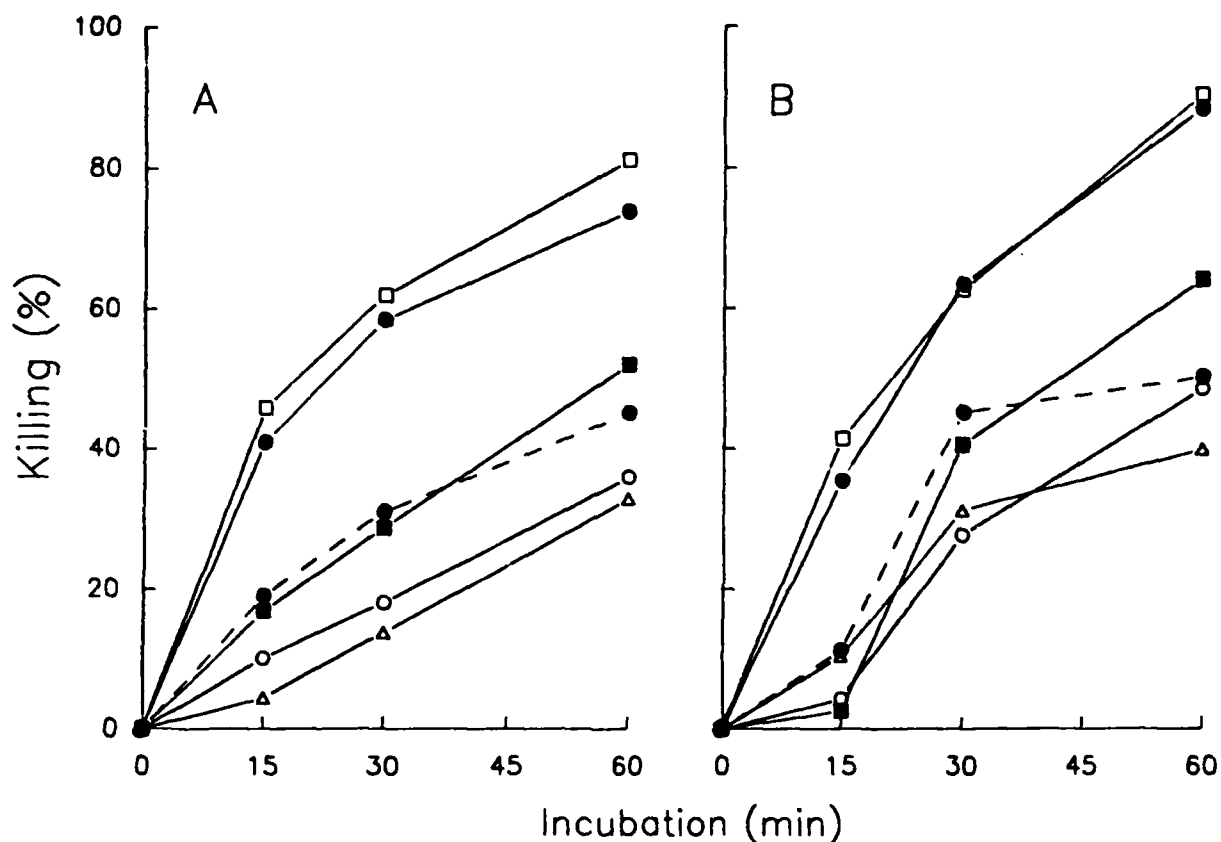
Figure 5. Effect of various concentrations of NSAIDs on the bactericidal activity of PMNs from thermally injured animals. PMNs harvested from injured animals at one day postburn were preincubated for 15 min at room temperature with piroxicam (A) or indomethacin (B) at the following concentrations before addition of the assay: 25 μM (O), 50 μM (■), 100 μM (●). The dashed line shows 100 μM piroxicam or indomethacin added to the PMNs without preincubation before assay. Untreated PMNs from injured animals (△) and normal animals (□) were also assayed. Data are from a representative experiment run in duplicate.

19

cells was measured in the presence of increasing concentrations of purified PGE_1 or PGE_2. Addition of PGE_1 (source, Advanced Magnetics) to the assays inhibited the bactericidal activity of PMNs from injured animals that had been treated with piroxicam in vitro (Fig. 6) or in vivo (Fig. 7). At a concentration of 5 μM PGE_1, bactericidal activity was reduced to the level observed before treatment of the PMNs with piroxicam. In contrast to the results obtained with PGE_1, concentrations of 1 or 5 μM PGE_2 (source, Advanced Magnetics) had no effect on the bactericidal activity of PMNs from injured animals treated with piroxicam in vitro or in vivo (data not shown). The same PGE_2 preparation also did not inhibit the bactericidal activity of PMNs from normal animals, whereas dose-dependent inhibition of the bactericidal activity of normal PMNs was observed with PGE_1 (Fig. 8). Similar results were obtained with a second preparation of purified PGE_2 from a different source (i.e., Upjohn Diagnostics; data not shown). These results supported involvement of PGE_1 but not PGE_2 in the bactericidal defect of PMNs in the injured animals.

Effect of cAMP and theophylline on the bactericidal activity of PMNs. PGE_1 has been shown to inhibit various functions of PMNs by raising intracellular cAMP [16-18,20]. If this mechanism was responsible for the observed inhibitory effects of PGE_1 on PMN bactericidal activity, then similar effects should be achieved with other agents that increase cAMP and with cAMP itself. To test this hypothesis, PMNs from injured animals were treated with piroxicam in vitro or in vivo, and the bactericidal activity of these cells was measured in the presence of cAMP, dibutyryl cAMP, or theophylline. Like PGE_1, cAMP, dibutyryl cAMP, and theophylline markedly reduced the bactericidal activity of PMNs from injured animals treated with piroxicam in vitro or in vivo (Fig. 9). These agents also decreased the bactericidal activity of normal PMNs (Fig. 10). cAMP and dibutyryl cAMP

20

Figure 6. Effect of PGE_1 on the bactericidal activity of PMNs from thermally injured animals treated in vitro with piroxicam. PMNs harvested from injured animals at one (A) or two (B) days postburn were preincubated for 15 min at room temperature with 100 μM piroxicam or were untreated. PMNs treated with piroxicam were assayed together with 0.5 μM PGE_1 (▲), 1 μM PGE_1 (O), 5 μM PGE_1 (■), or buffer (●). Untreated PMNs from injured animals (△) and normal animals (□) were assayed in the absence of PGE_1. Data are from a representative experiment run in duplicate.
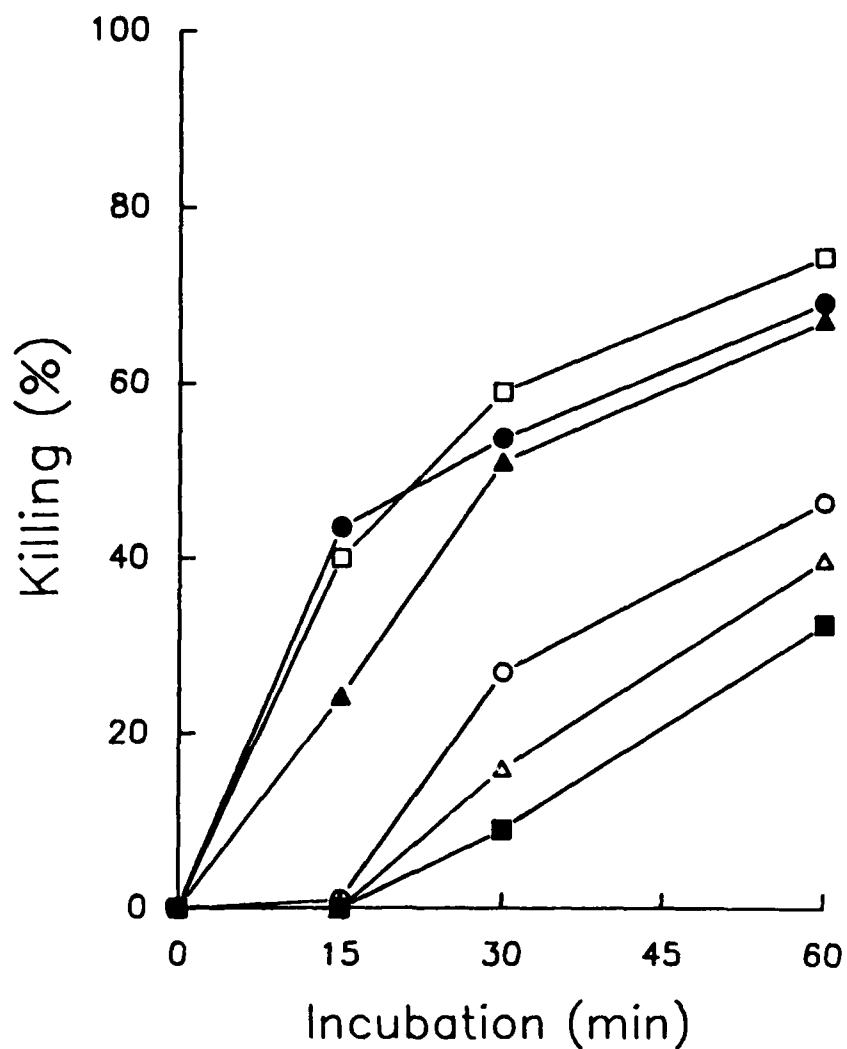
Figure 7. Effect of PGE_1 on the bactericidal activity of PMNs from thermally injured animals treated in vivo with piroxicam. PMNs were harvested at one day postburn from injured animals treated parenterally with piroxicam and from untreated animals. PMNs from animals treated with piroxicam were assayed together with 0.5 μM PGE_1 (▲), 1 μM PGE_1 (O), 5 μM PGE_1 (■), or buffer (●). PMNs from untreated injured animals (△) and normal animals (□) were assayed in the absence of PGE_1. Data are from a representative experiment run in duplicate.
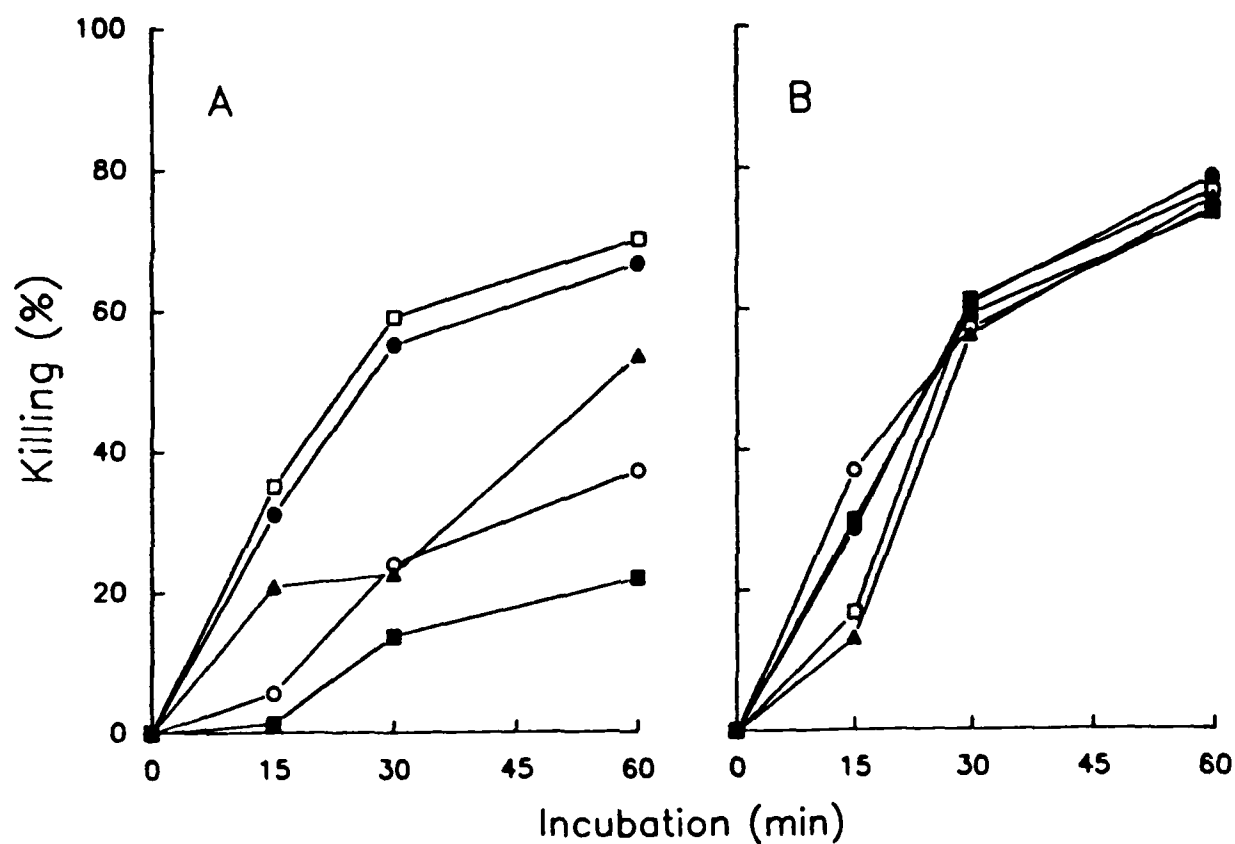
Figure 8. Effect of PGE$_1$ and PGE$_2$ on the bactericidal activity of PMNs from normal animals. Normal PMNs were assayed together with PGE$_1$ (A) or PGE$_2$ (B) at the following concentrations: 0.05 μM ($\bullet$), 0.5 μM ($\blacktriangle$), 1 μM (O), 5 μM ($\blacksquare$). Normal PMNs were also assayed in the absence of PGE$_1$ or PGE$_2$ ($\square$). Data are from a representative experiment run in duplicate.
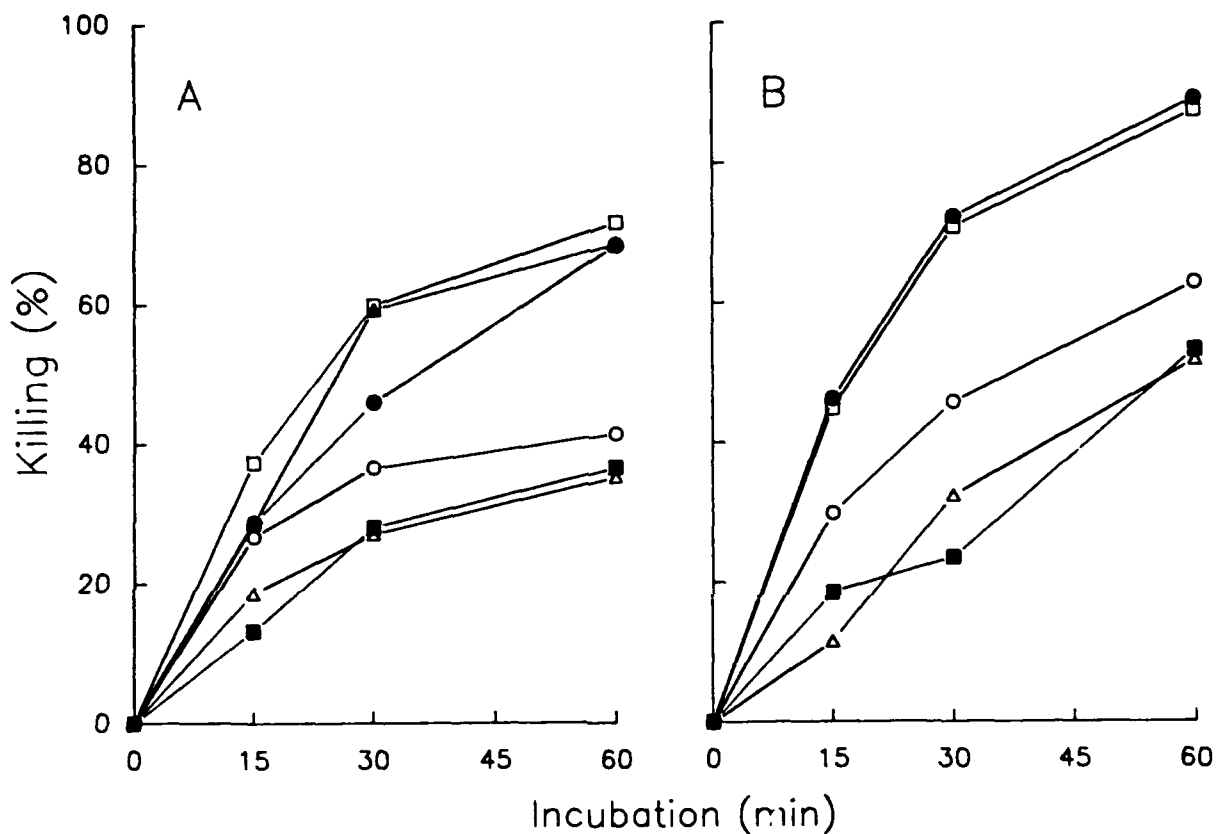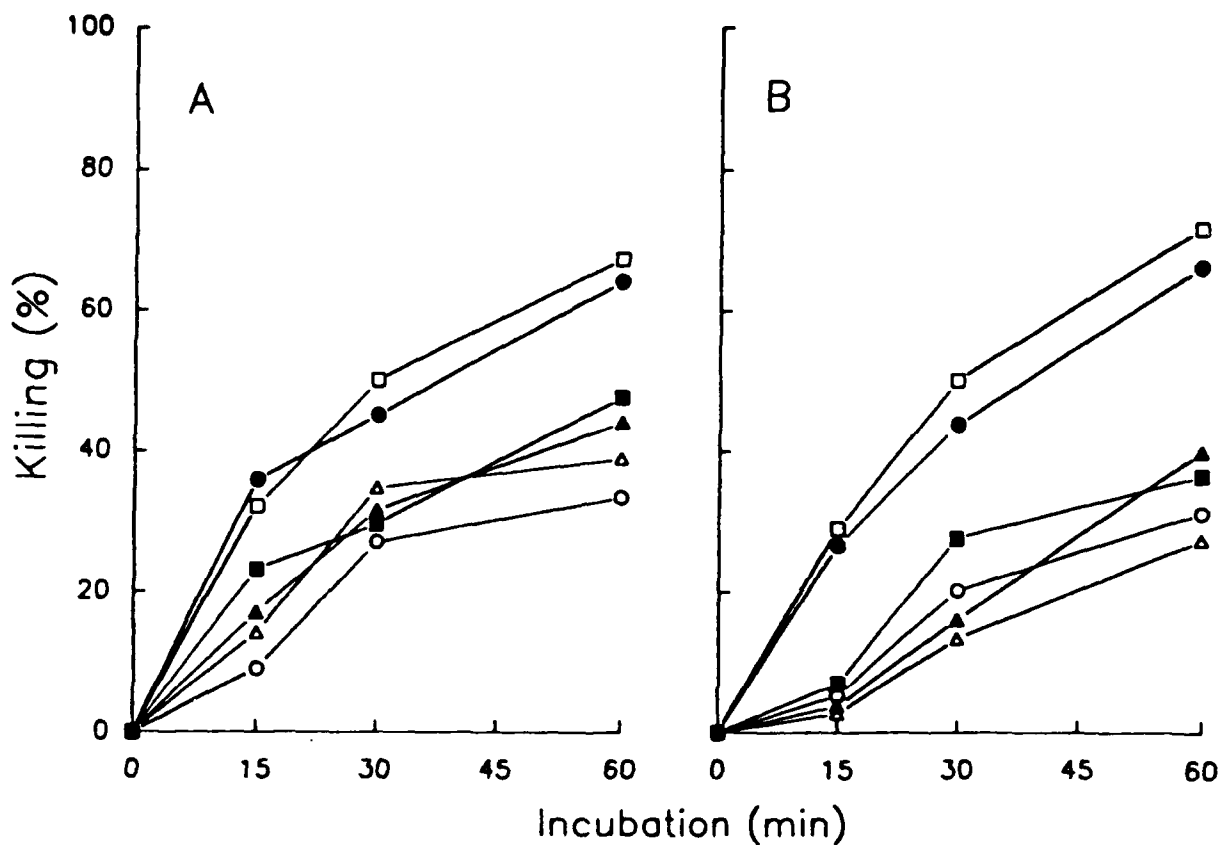
Figure 9. Effect of cAMP and theophylline on the bactericidal activity of PMNs from thermally injured animals treated in vitro or in vivo with piroxicam. PMNs harvested from injured animals at one day postburn were treated in vitro (A) or in vivo (B) with piroxicam or were untreated. PMNs treated with piroxicam were assayed together with 10 mM cAMP (■), 10 mM dibutyryl cAMP (O), 1 mM theophylline (▲), or buffer (●). Untreated PMNs from injured animals (△) and normal animals (□) were assayed in the absence of cAMP and theophylline. Data are from a representative experiment run in duplicate.

24

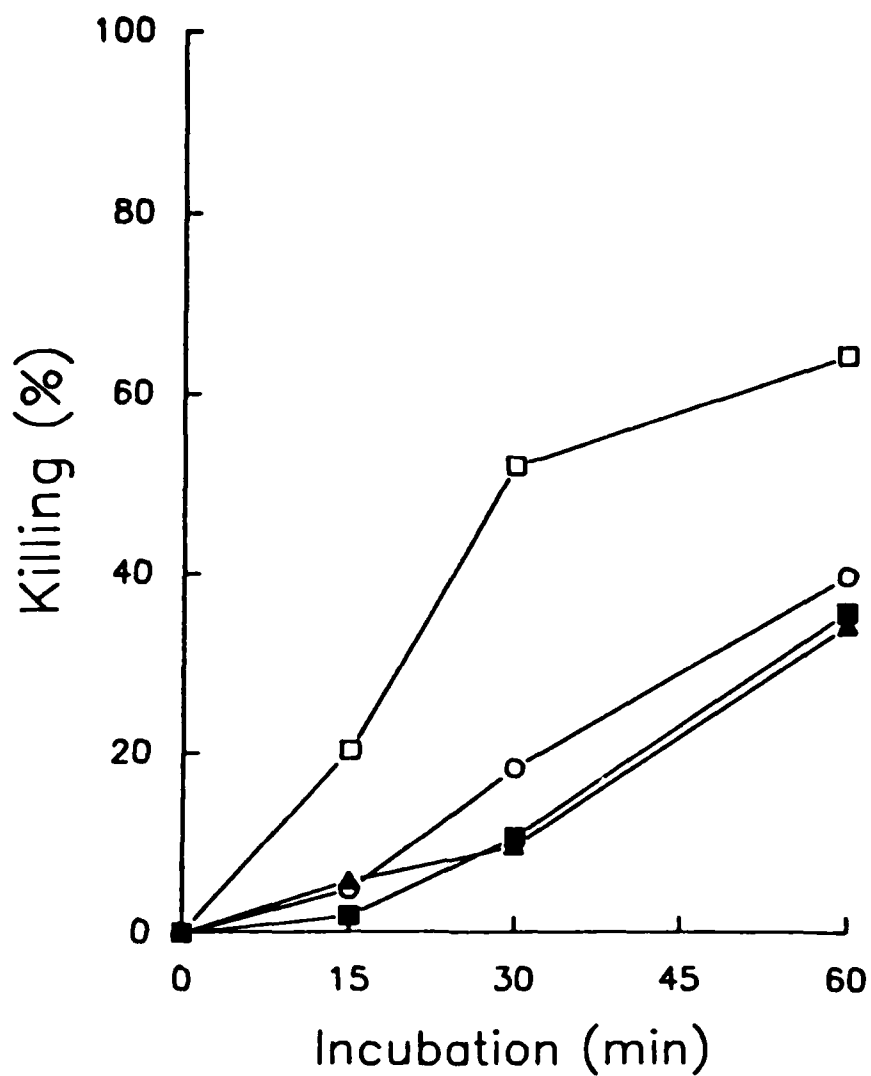*Figure 10.* Effect of cAMP and theophylline on the bactericidal activity of PMNs from normal animals. Normal PMNs were assayed alone (□) and together with 10 mM cAMP (■), 10 mM dibutyryl cAMP (○), or 1 mM theophylline (▲). Data are from a representative experiment run in duplicate.

were both effective in reducing the bactericidal activity of PMNs. Dibutyryl cAMP is more accessible for entry into cells than cAMP; however, both compounds appear to be capable of entering PMNs at the high concentrations used in our study. These results suggested that PGE_1 inhibited PMN bactericidal activity in the preceding experiments by raising intracellular cAMP.

cAMP accumulation in PMNs under various conditions. To further investigate the relationship between PMN bactericidal activity and intracellular cAMP, the cAMP content of PMNs from injured and normal animals was compared. In addition, the effect of treatment with NSAIDs and cAMP activators on cAMP content was determined under conditions identical to those used in the bactericidal assays. The cAMP content was approximately fivefold higher in PMNs from injured animals than in PMNs from normal animals (Table I). Treatment of PMNs from injured animals with NSAIDs in vitro or in vivo markedly reduced cAMP. When PMNs from injured animals were treated with piroxicam in vivo and then incubated with PGE_1, the cAMP content increased. Major increases in cAMP content were also observed upon treatment of normal PMNs with PGE_1 or theophylline, but not with PGE_2. These results suggested that NSAIDs and the cAMP activators modulated PMN bactericidal activity through effects on cAMP.

Relationship between cAMP accumulation and PGE_1 production by PMNs. To determine if the elevation of cAMP in PMNs from injured animals was associated with increased production of PGE_1 by these cells, spontaneous production of PGE_1 by PMNs from injured and normal animals was compared. The effect of NSAIDs on PGE_1 production by PMNs from injured animals was also determined. PMNs from injured animals produced approximately threefold more PGE_1 than PMNs from normal animals (Table II). Treatment of PMNs from injured animals with NSAIDs in vitro or in vivo reduced PGE_1 production to the level observed with normal PMNs. These results suggested that the

26

TABLE I

cAMP content of PMNs under various conditions[a]

Source of PMNs	Treatment of PMNs		cAMP content (pmol/10^7 cells)
	NSAID	cAMP activator	
Injured animals (day 1 postburn)	None	None	109.3 ± 10.1
	Indomethacin (in vitro)	None	44.8 ± 3.0
	Ibuprofen (in vitro)	None	45.0 ± 2.0
	Piroxicam (in vitro)	None	46.8 ± 0.8
	Piroxicam (in vivo)	None	29.3 ± 0.3
	Piroxicam (in vivo)	PGE_1	92.0 ± 2.6
Normal animals	None	None	19.8 ± 1.1
	None	PGE_1	97.0 ± 5.9
	None	PGE_2	40.5 ± 2.9
	None	Theophylline	144.3 ± 5.4

[a]PMNs were first preincubated for 15 min at room temperature with 100 μM NSAID or were treated with piroxicam in vivo as described in the *Methods* section. PGE_1 (5 μM), PGE_2 (5 μM), or theophylline (1 mM) were then added to the PMNs; under these conditions, the cells were incubated for 2 min at 37°C before boiling and sonication. Data are from a representative experiment. Data are mean $\pm$ SEM; n, 4.

TABLE II

Effect of NSAIDs on PGE_1 production by PMNs[a]

Source of PMNs	Treatment of PMNs	PGE_1 production (pg/10^7 cells)
Injured animals (day 1 postburn)	None	$19,032 \pm 196$
	Indomethacin (in vitro)	$5,841 \pm 304$
	Ibuprofen (in vitro)	$6,139 \pm 359$
	Piroxicam (in vitro)	$6,114 \pm 316$
	Piroxicam (in vivo)	$4,551 \pm 433$
Normal animals	None	$6,490 \pm 146$

[a]PMNs were first preincubated for 15 min at room temperature with 100 μM NSAID or were treated with piroxicam in vivo as described in the *Methods* section. PMNs were then incubated for 15 min at 37°C in the absence of exogenous PMN activators, and the amount of PGE_1 released into the supernatants was determined. Data are from a representative experiment. Data are mean $\pm$ SEM; n, 4.

increased production of PGE_1 by PMNs from injured animals contributed to the elevation of cAMP within these cells.

To further determine the relationship between cAMP content, PGE_1 production, and bactericidal activity, these parameters were measured in PMNs harvested from injured animals at one, two, four, and seven days postburn. Bactericidal activity was restored to normal during four to seven days postburn (Table III). cAMP content and PGE_1 production were reduced concomitantly during this period. These results provided additional evidence that the bactericidal defect of PMNs induced by thermal injury was related to elevation of cAMP and that PGE_1 contributed to this phenomenon.

Postburn changes in cAMP content, PGE_1 production, and bactericidal activity of peritoneal exudate PMNs. The relationship between cAMP content, PGE_1 production, and bactericidal activity was also investigated using PMNs harvested from peritoneal exudates. The trend of results using PMNs from this source (Table IV) was similar to that obtained using peripheral PMNs (Table III), except the extent of elevation of cAMP and PGE_1 production by peritoneal exudate PMNs was less than that observed with peripheral PMNs (compare Tables III and IV). This difference in results may be related to the greater purity of the peritoneal exudate PMN preparations (>95% pure) as compared with the peripheral PMN preparations ($\geq$85% pure). Some of the PGE produced by the peripheral PMN preparations may be derived from contaminating mononuclear cells and/or possibly also platelets, which have a more active cyclooxygenase system. The additional PGE produced by these cells may boost cAMP levels in the PMNs.

Discussion and Conclusions

Previous studies have suggested that cAMP plays an important role in the negative feedback control of PMN effector functions [16-18,20,29-32].

TABLE III

Natural changes in cAMP content, PGE_1 production, and bactericidal

activity of PMNs during the first week postburn[a]

Source of PMNs	Days postburn	cAMP content (pmol/10^7 cells)	PGE_1 production (pg/10^7 cells)	Killing (%)		
				15 min	30 min	60 min
Injured animals	1	114.0 ± 6.5	20,588 ± 293	0	8	40
	2	94.5 ± 0.9	18,416 ± 772	1	19	31
	4	70.3 ± 5.8	16,474 ± 745	15	32	69
	7	46.1 ± 5.7	12,491 ± 397	20	53	64
Normal animals		19.6 ± 1.2	8,288 ± 498	26	53	65

[a]Bactericidal activity was assayed in duplicate. Other data are mean ± SEM; n, 4.

TABLE IV

Postburn changes in cAMP content, PGE_1 production, and bactericidal

activity of peritoneal exudate PMNs[a]

Source of PMNs	Days postburn	cAMP content (pmol/10^7 cells)	PGE_1 production (pg/10^7 cells)	Killing (%)		
				15 min	30 min	60 min
Injured animals	1	79.8 ± 2.7	11,956 ± 301	15 ± 6	23 ± 2	34 ± 4
	2	60.0 ± 6.5	10,836 ± 684	16 ± 5	30 ± 5	40 ± 4
	4	41.8 ± 2.5	9,974 ± 718	23 ± 4	31 ± 5	47 ± 5
	7	20.3 ± 2.4	7,973 ± 355	31 ± 5	44 ± 4	58 ± 2
Normal animals		10.3 ± 0.5	5,747 ± 254	36 ± 2	56 ± 2	71 ± 1

[a]Data are mean ± SEM; n, 3 (bactericidal activity) or n, 4 (cAMP content and PGE_1

production).

These studies have shown that exposure of normal PMNs in vitro to high concentrations of cAMP or to agents that markedly elevate intracellular cAMP diminishes chemotaxis, phagocytosis, degranulation, oxidative metabolism, and iodination. The present investigation is the first to demonstrate the natural occurrence of this mechanism in vivo in the heightened inflammatory state associated with thermal injury. Using a guinea pig model of thermal injury, we demonstrated that peripheral and peritoneal exudate PMNs from injured animals had a marked elevation of cAMP as compared with similar PMN preparations from normal animals. This elevation of cAMP was associated with defective bactericidal activity against $P.$ $aeruginosa$. The bactericidal defect was corrected by treating peripheral PMNs from the injured animals with NSAIDs in vitro or in vivo, with concomitant reduction in the cAMP content of these cells. When cAMP was reelevated by treating the PMNs with PGE_1, theophylline, or with cAMP itself, bactericidal activity was again reduced to an abnormally low level. Thus, by artificially raising and lowering the cAMP content of PMNs from the injured animals, we were able to inversely modulate bactericidal activity.

Natural changes in cAMP content of PMNs from the injured animals and associative changes in bactericidal activity during the first week postburn were also studied. cAMP in these cells was maximally increased and bactericidal activity was maximally reduced at one day postburn. By four days postburn, the cAMP content had declined and, by seven days postburn, a major reduction in cAMP was observed. Bactericidal activity gradually increased during this time period and was restored to an approximately normal level by seven days postburn. The extent of the reduction in the cAMP content at seven days postburn was equivalent to that observed when PMNs obtained early after injury were treated with NSAIDs in vitro. These observations taken together with those described above provide strong support

32

for the hypothesis that the bactericidal defect of PMNs induced by thermal injury is related to a marked elevation of cAMP.

Our study also investigated the mechanisms responsible for the increase in cAMP in PMNs from the injured animals. Several pieces of evidence were obtained suggesting that PGE_1, the major E type prostaglandin produced by activated PMNs [33], plays a significant role in this phenomenon. First, peripheral and peritoneal exudate PMNs from the injured animals produced considerably more PGE_1 than similar PMN preparations from normal animals when incubated in vitro in the absence of exogenous PMN activators. Secondly, spontaneous PGE_1 production and the cAMP content of these cells were reduced concomitantly as bactericidal activity was restored to normal during four to seven days postburn and also under conditions in which peripheral PMNs obtained early after injury were treated in vitro or in vivo with NSAIDs. Thirdly, a concentration of 100 μM NSAID was required to normalize the bactericidal activity of these cells in vitro; this drug concentration was also required to effectively reduce the cAMP content and PGE_1 production by these cells (data not shown).

PGE_2 was not found to play a major role in the elevation of cAMP in PMNs from the injured animals. Purified PGE_2 from two different sources had no effect on the bactericidal activity of normal PMNs or PMNs from injured animals that had been treated in vitro or in vivo with piroxicam; bactericidal activity of PGE_2-treated cells was equal to that of normal PMNs. In addition, incubation of normal PMNs with purified PGE_2 at a concentration of 5 μM caused only a minimal increase in cAMP. Thus, on a molar basis, PGE_2 was considerably less efficient than PGE_1 in raising the cAMP content of PMNs, and this presumably explains why it had no effect on bactericidal activity.

Ishitoya and Takenawa have recently demonstrated that PGE_1 acts syner-gistically with substances that activate PMNs to increase and maintain intracellular cAMP at very high levels [34]. This mechanism may be in-volved in the elevation of cAMP observed in our study. A number of media-tors produced in response to thermal injury have the potential to increase cAMP in PMNs. Our previous studies have shown that complement cleavage fragments are present in circulation in injured animals early after injury [14], and Moore et al. have suggested that these fragments contribute to the activation of PMNs in injured patients [12]. Substances released from damaged tissue and bacterial products formed as a result of bacterial colonization of the burn wound may also be involved in PMN activation and the associated increase in cAMP. Catecholamines and glucocorticoids are thought to be responsible for the hypermetabolic response to thermal injury [35,36], and these substances are known to have profound effects on cAMP accumulation. Like PGE_1, catecholamines increase cAMP in PMNs by acting as adenyl cyclase agonists [37], whereas glucocorticoids potentiate the effects of these agents [20].

Our study did not prove that NSAIDs lowered the cAMP content of PMNs from the injured animals entirely through inhibition of PGE_1 production. Other mechanisms may also be involved. Concentrations of NSAIDs as high as those used in our in vitro studies have been shown to inhibit the activa-tion of normal PMNs [38-40]. It has been proposed that NSAIDs interfere with the triggering of cellular processes by blocking the interaction be-tween the G protein and the receptor for the ligand [40]. However, this mechanism would not explain our finding that NSAIDs corrected the bacteri-cidal defect of PMNs from the injured animals when employed in vitro, since activation of these cells had presumably already occurred in vivo. There is little known about the effects of NSAIDs on PMNs after they have under-

34

gone activation; however, our results suggest that these drugs modulate cAMP during this phase.

One of the most significant observations of our study is that the bactericidal defect of PMNs induced by thermal injury is reversible. It has been previously postulated that the depression in PMN effector functions associated with thermal injury reflects a state of irreversible cell exhaustion following maximal stimulation. Our study indicates that this is not correct with respect to bactericidal activity. However, it is possible that other effector functions of these cells, such as chemotaxis, may be irreversibly altered by thermal injury, even though cAMP accumulation in these cells can be effectively reduced by pharmacologic means. The reduction in cAMP would facilitate reversal of microtubular disassembly [41]. However, the depression in chemotaxis is also associated with depletion of PMN specific granules, increased expression of the complement receptors, CR1 and CR3, and internalization of C5a receptors [9,10,12]. These events may not be reversible.

The elevation of cAMP in PMNs produced in response to injury and the associated depression in effector functions may have been designed teleologically to protect the host from the harmful consequences of PMN activation. At present, it is not known whether therapy with NSAIDs by increasing bactericidal activity would increase resistance against infection, or whether this therapy would allow for continued PMN activation with associated adverse effects on the host. Our observation that piroxicam was capable of reducing the cAMP content of PMNs and normalizing bactericidal activity in vivo despite ongoing inflammation suggests that this drug may be capable of beneficially modulating PMN activation in an inflammatory environment. The clinical application of these findings is dependent on understanding the mechanisms responsible for and the reversibility of other PMN alterations induced by severe injury.

Reference List

1. Mason, A. D., Jr., A. T. McManus, and B. A. Pruitt, Jr. 1986. Association of burn mortality and bacteremia. A 25-year review. *Arch. Surg.* 121:1027-1031.

2. Bjornson, A. B., and H. S. Bjornson. 1984. Theoretical interrelationships among immunologic and hematologic sequelae of thermal injury. *Rev. Infect. Dis.* 6:704-714.

3. Quie, P. G. 1987. The white cells: use of granulocyte transfusions. *Rev. Infect. Dis.* 9:189-193.

4. Alexander, J. W., and J. L. Meakins. 1972. A physiological basis for the development of opportunistic infections in man. *Ann. Surg.* 176:273-287.

5. Warden, G. D., A. D. Mason, Jr., and B. A. Pruitt, Jr. 1974. Evaluation of leukocyte chemotaxis in vitro in thermally injured patients. *J. Clin. Invest.* 54:1001-1004.

6. Alexander, J. W., C. K. Ogle, J. D. Stinnett, and B. G. MacMillan. 1978. A sequential, prospective analysis of immunologic abnormalities and infection following severe thermal injury. *Ann. Surg.* 188:809-816.

7. Alexander, J. W., J. D. Stinnett, C. K. Ogle, J. D. Ogle, and M. J. Morris. 1979. A comparison of immunologic profiles and their influence on bacteremia in surgical patients with a high risk of infection. *Surgery* 86:94-104.

8. Heck, E., M. A. Edgar, J. L. Hunt, and C. R. Baxter. 1980. A comparison of leukocyte function and burn mortality. *J. Trauma* 20:75-77.

9. Davis, J. M., P. Dineen, and J. I. Gallin. 1980. Neutrophil degranulation and abnormal chemotaxis after thermal injury. *J. Trauma* 124:1467-1471.

10. Solomkin, J. S., R. D. Nelson, D. E. Chenoweth, L. D. Solem, and R. L. Simmons. 1984. Regulation of neutrophil migratory function in burn injury by complement activation products. *Ann. Surg.* 200:742-746.

11. Duque, R. E., S. H. Phan, J. L. Hudson, G. O. Till, and P. A. Ward. 1985. Functional defects in phagocytic cells following thermal injury. *Am. J. Pathol*. 118:116-127.

12. Moore, F. D., Jr., C. Davis, M. Rodrick, J. A. Mannick, and D. T. Fearon. 1986. Neutrophil activation in thermal injury as assessed by increased expression of complement receptors. *N. Engl. J. Med*. 314:948-953.

13. Maderazo, E. G., C. L. Woronick, S. D. Albano, S. P. Breaux, and R. M. Pock. 1986. Inappropriate activation, deactivation, and probable auto-oxidative damage as a mechanism of neutrophil locomotory defect in trauma. *J. Infect. Dis*. 154:471-477.

14. Bjornson, A. B., H. S. Bjornson, R. W. Knippenberg, and J. S. Cardone. 1986. Temporal relationships among immunologic alterations in a guinea pig model of thermal injury. *J. Infect. Dis*. 153:1098-1107.

15. Bjornson, A. B., R. W. Knippenberg, and H. S. Bjornson. 1988. Nonsteroidal anti-inflammatory drugs correct the bactericidal defect of polymorphonuclear leukocytes in a guinea pig model of thermal injury. *J. Infect. Dis*. 157:959-967.

16. Zurier, R. B., G. Weissmann, S. Hoffstein, S. Kammerman, and H. H. Tai. 1974. Mechanisms of lysosomal enzyme release from human leukocytes. II. Effects of cAMP and cGMP, autonomic agonists, and agents which affect micro-tubule function. *J. Clin. Invest*. 53:297-309.

17. Rivkin, I., J. Rosenblatt, and E. L. Becker. 1975. The role of cyclic AMP in the chemotactic responsiveness and spontaneous motility of rabbit peritoneal neutrophils. The inhibition of neutrophil movement and the elevation of cyclic AMP levels by catecholamines, prostaglandins, theophylline and cholera toxin. *J. Immunol*. 115:1126-1134.

18. Lehmeyer, J. E., and R. B. Johnston, Jr. 1978. Effect of anti-inflammatory drugs and agents that elevate intracellular cyclic AMP on the release of toxic oxygen metabolites by phagocytes: Studies in a model of tissue-bound IgG. *Clin. Immunol. Immunopathol.* 9:482-490.

19. O'Flaherty, J. T., D. L. Kreutzer, and P. A. Ward. 1979. Effect of prostaglandins E_1, E_2 and $F_{2\alpha}$ on neutrophil aggregation. *Prostaglandins* 17:201-210.

20. Marone, G., L. L. Thomas, and L. M. Lichtenstein. 1980. The role of agonists that activate adenylate cyclase in the control of cAMP metabolism and enzyme release by human polymorphonuclear leukocytes. *J. Immunol.* 125:2277-2283.

21. Fantone, J. C., S. L. Kunkel, and P. A. Ward. 1981. Suppression of human polymorphonuclear function after intravenous infusion of prostaglandin E_1. *Prostaglandins Med.* 7:195-198.

22. Fantone, J. C., W. A. Marasco, L. J. Elgas, and P. A. Ward. 1983. Anti-inflammatory effects of prostaglandin E_1: In vivo modulation of the formyl peptide chemotactic receptor on the rat neutrophil. *J. Immunol.* 130:1495-1497.

23. Fantone, J. C., and D. A. Kinnes. 1983. Prostaglandin E_1 and prostaglandin I_2 modulation of superoxide production by human neutrophils. *Biochem. Biophys. Res. Commun.* 113:506-512.

24. McCarthy, J. P., R. S. Bodroghy, P. B. Jahrling, and P. Z. Sobocinski. 1980. Differential alterations in host peripheral polymorphonuclear leukocyte chemiluminescence during the course of bacterial and viral infections. *Infect. Immun.* 30:824-831.

25. Zimmerli, W., B. Seligmann, and J. I. Gallin. 1986. Exudation primes human and guinea pig neutrophils for subsequent responsiveness to the chemotactic peptide N-formylmethionylleucylphenylalanine and increases

complement component C3bi receptor expression. *J. Clin. Invest.* 77:925-933.

26. Michell, R. H., S. J. Pancake, J. Noseworth, and M. L. Karnovksy. 1969. Measurement of rates of phagocytosis: the use of celular monolayers. *J. Cell Biol.* 40:216.

27. Bjornson, A. B., P. I. Magnafichi, R. D. Schreiber, and H. S. Bjornson. 1987. Opsonization of *Bacteroides* by the alternative complement pathway reconstructed from isolated plasma proteins. *J. Exp. Med.* 164:777-798.

28. Powell, W. S. 1980. Rapid extraction of oxygenated metabolites of arachidonic acid from biological samples using octadecylsilyl silica. *Prostaglandins* 20:947-957.

29. May, C. D., B. B. Levine, and G. Weissmann. 1970. Effects of compounds which inhibit antigenic release of histamine and phagocytic release of lysosomal enzyme on glucose utilization by leukocytes in humans. *Proc. Soc. Exp. Biol. Med.* 133:758-763.

30. Stolc, V. 1972. Regulation of iodine metabolism in human leukocytes by adenosine 3',5'-monophosphate. *Biochem. Biophys. Acta.* 264:285-288.

31. Cox, J. P., and M. L. Karnovsky. 1973. The depression of phagocytosis by exogenous cyclic nucleotides, prostaglandins, and theophylline. *J. Cell Biol.* 59:480-490.

32. Bessler, H., R. Gilgal, M. Djaldetti, and I. Zahavi. 1986. Effect of pentoxifylline on the phagocytic activity, cAMP levels, and superoxide anion production by monocytes and polymorphonuclear cells. *J. Leukocyte Biol.* 40:747-754.

33. Zurier, R. B., and D. M. Sayadoff. 1975. Release of prostaglandins from human polymorphonuclear leukocytes. *Inflammation* 1:93-101.

34. Ishitoya, J., and T. Takenawa. 1987. Potentiation of PGE_1-induced increase in cyclic AMP by chemotactic peptide and Ca^{2+} ionophore through calmodulin-dependent processes. *J. Immunol.* 138:1201-1207.

35. Wilmore, D. W., J. M. Long, A. D. Mason, Jr., R. W. Skreen, and B. A. Pruitt, Jr. 1974. Catecholamines: Mediator of the hypermetabolic response to thermal injury. *Ann. Surg.* 180:653-668.

36. Vaughan, G. M., R. A. Becker, J. P. Allen, C. W. Goodwin, Jr., B. A. Pruitt, Jr., and A. D. Mason, Jr. 1982. Cortisol and corticotrophin in burned patients. *J. Trauma* 22:263-272.

37. Bourne, H. R., and K. L. Melmon. 1971. Adenyl cyclase in human leukocytes: Evidence for activation by separate beta adrenergic and prostaglandin receptors. *J. Pharmacol. Exp. Therap.* 178:1-7.

38. Davies, J., K. Sheppard, and J. Fletcher. 1984. Inhibition of human neutrophil secondary granule discharge by antiinflammatory agents. *Inflammation* 8:343-351.

39. Abramson, S., H. Korchak, R. Ludewig, H. Edelson, K. Haines, R. I. Levin, R. Herman, L. Rider, S. Kimmel, and G. Weissmann. 1985. Modes of action of aspirin-like drugs. *Proc. Natl. Acad. Sci. USA.* 82:7227-7231.

40. Weissmann, G. 1987. Pathogenesis of inflammation. Effects of the pharmacological manipulation of arachidonic acid metabolism on the cytological response to inflammatory stimuli. *Drugs* 33 (Suppl. 1):28-37.

41. Gillespie, E. 1971. Colchicine binding in tissue slices. Decrease by calcium and biphasic effect of adenosine-3',5'-monophosphate. *J. Cell Biol.* 50:544-549.

DISTRIBUTION LIST

4 Copies   Commander
Letterman Army Institute of Research (LAIR)
Building 1110
ATTN: SGRD-ULZ-RC
Presidio of San Francisco, CA   94129-6815

1 Copy   Commander
U.S. Army Medical Research and Development Command
ATTN: SGRD-RMI-S
Fort Detrick, Frederick, Maryland 21701-5012

2 Copies   Defense Technical Information Center (DTIC)
ATTN: DTIC-FDAC
Cameron Station
Alexandria, VA 22304-6145

1 Copy   Dean
School of Medicine
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814-4799

1 Copy   Commandant
Academy of Health Sciences, U.S. Army
ATTN: AHS-CDM
Fort Sam Houston, TX 78234-6100